Gypsy Moth News

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Gypsy Moth News

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In this Issue

Bacillus thuringiensis — What it is and how it works

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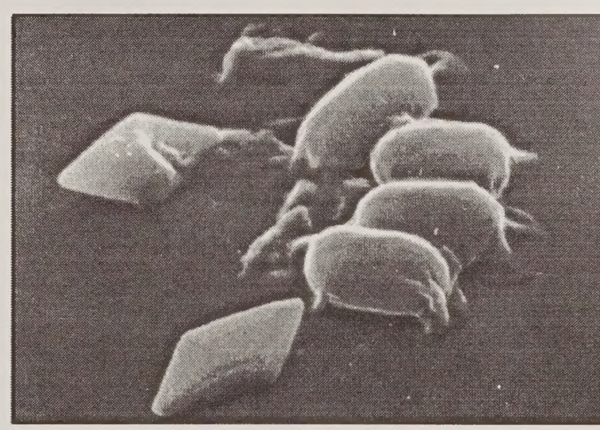
Bacillus thuringiensis (*B.t.*) occurs naturally in numerous species of agricultural and forest insects and is a natural component of the soil microbiota worldwide. Typically, they are rod-shaped, form a spore, and are motile. Unique to this species, at the time of sporulation, they form a protein crystal next to the spore. These crystals are unique crystalline endotoxins.



Crystals (left), *B.t.* spores (right).
Photograph by John Podgwaite, Forest Service

Caterpillars feed on leaves that have been treated with *B.t.* The spores and crystals enter the alkaline stomach where the crystals dissolve. Within 24 to 48 hours the dissolved crystals have caused the stomach wall to break down and spores begin to invade the body. During the next 48 to 96 hours, the spores completely invade the body and germinate. The caterpillar dies from the combined effects of starvation and tissue damage.

The new bacteria living in the caterpillar are unable to form spores or crystals; therefore, it dies and decays along with the caterpillar, avoiding any insecticide buildup in the environment.

Bacillus thuringiensis — for Managing Gypsy Moth*

Richard Reardon[1], Norman Dubois[2], and Winfred McLane[3]

The *Bacillus thuringiensis* Berliner group of bacteria (commonly referred to as *B.t.*) is receiving increasing attention for use in integrated pest management programs for agricultural and forest insect pests and insect vectors of human and other mammalian transmissible disease. Taxonomically, these entomopathogenic (causing disease in insects) bacteria are in the family *Bacillaceae* and are members of the genus *Bacillus*. Typically, they are rod-shaped, form a spore, and are motile by flagellae (whip-like appendages). In addition and unique to this species, they form a protein crystal next to the spore at the time of sporulation. *B. thuringiensis* occurs naturally in numerous species of agricultural and forest insects and is a component of the soil microbiota worldwide (Martin and Travers 1989). Many different strains of *B.t.* have been isolated from soils; however, most strains used in commercial production of microbial insecticides have been isolated from diseased insects (DeLucca et al. 1981).

Bacillus thuringiensis was first isolated from diseased silkworm, *Bombyx mori* (L.), larvae in Japan in 1901 by Ishiwata who named it *Bacillus sotto*. In 1911, a German entomologist named Berliner isolated another variety of this bacterium from diseased Mediterranean flour moths, Ephestia (=*Anagasta*) *kuehniella* (Zeller), that were found in stored grain in Thuringia. In 1915, he named it *Bacillus thuringiensis*. Berliner recorded the first scientific description of the bacterium (Ishiwata did not formally describe the organism he found), and is credited with naming it (Beegle and Yamamoto, 1992). This culture (Berliner strain of variety *thuringiensis*) was lost, and in 1927 Mattes reisolated the same organism from the same host as did Berliner. Mattes' isolate was widely distributed in most of the early commercial *B.t.*-based products, and to date, it is the representative strain for the type species of these crystal-forming bacteria.

Through the research and promotional efforts of E.A. Steinhaus in the early 1950's, development of *B. thuringiensis* var. *thuringiensis* proceeded quickly and led to commercial production and extensive research. Kurstak, in 1962, isolated another variety of *B. thuringiensis* that was effective primarily against Lepidoptera and named it *kurstaki*. In 1970, Dulmage isolated another more potent strain of this variety from diseased mass-reared pink bollworm, *Pectinophora gossypiella* (Saunders), larvae and coded it the HD-1 strain (Dulmage 1970). This strain, often referred to by its acronym "*B.t.k.*", became commercially available through Abbott Laboratories as Dipel in the early 1970's. Since this strain is active and more potent than previous strains against numerous lepidopteran species, it is used today for production of most formulations of *B.t.* that are used to control defoliating Lepidoptera in North America. What began in the 1950's as a collection of less than a dozen *B.t.* strains, now has grown to over 1,000 strains of different varieties maintained at the *Bacillus thuringiensis* Culture Repository at the Northern Regional Research Laboratory (N.R.R.L.) of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), at Peoria, Illinois. In addition, a large number of cultures and variants developed through genetic manipulations are held by industry.

First attempts to use *B. thuringiensis* for insect control took place in the late 1920's against the gypsy moth, *Lymantria dispar* (L.), in the northeastern United States (Metalnikov and Chorine 1929) and in the early 1930's against the European corn borer, *Ostrinia nubilalis* (Huber), in eastern Europe. The first commercial *B. thuringiensis* product, Sporeine, was available in 1938 in France (Entwistle et al. 1993). In the United States, the first commercial *B. thuringiensis* product, Thuricide (Pacific Yeast Products-Bioferm Corp.), became available for testing in 1958. In 1960, the United States Food and Drug Administration (FDA) granted a full exemption from residue tolerances, and the first formulated *B.t.* product was registered in 1961, under the trade name of Thuricide by the Pesticide Regulation Division of the USDA.

Since 1980, 1.7 million hectares (ha) (4.2 million acres) have been treated with *B. thuringiensis* var. *kurstaki* (*B.t.k.*) in the eastern United States as part of the Federal, State, and County Gypsy Moth Cooperative Suppression Program. During this interval, the use of *B.t.* against the North American gypsy moth (European strain introduced and established in the U.S. since the 1860's) ranged from a low of 6.4 percent of the total area treated with *B.t.* to a high of 79.5 percent in any single year (Machesky 1993). A wide range of aircraft types and spray equipment was used to apply various doses, rates, and formulations of *B.t.* Generally, one application was used in normal spray operations. However, two or three applications were commonly used in eradication efforts in Oregon and Utah against the North American gypsy moth, and in Washington and Oregon on approximately 200,000 ha against the Asian strain of gypsy moth. Also, multiple applications (2 to 3) of *B.t.* were used to eradicate an infestation of European, Asian, and hybrid strains of the gypsy moth on approximately 50,000 ha in eastern North Carolina. Between 1985 and 1990 in Ontario, Canada, 204,000 ha were treated with *B.t.* to control North American gypsy moth.

* Excerpt from Bacillus thuringiensis for Managing Gypsy Moth: A Review, FHM-NC-01-94

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B.t. usage by State - 1999

State/Site	Pesticide		Application Rate
UT	Foray 48B		24 BIU/acre
WV	Foray 48F		24 BIU/acre
VA	Thuricide 48LV		24 BIU/acre
NC	Thuricide 48LV		24 BIU/acre
Highlands Area (NC)	Foray 48F		24 BIU/acre
TN	Foray 48B		24 BIU/acre
NJ	Foray 48B		30 BIU/acre
WI	Foray 48F	90%	36 BIU/acre
	Foray 76B	10%	36+ BIU/acre
MD	Thuricide 48LV		24 BIU/acre
OH	Foray 76B		40 BIU/acre
PA	Foray 48F		24 FTU/acre
Raystown Lake, PA	Foray 48F		36 BIU/acre
MI	Thuricide 48LV		24 BIU/acre
	Foray 48F		24 BIU/acre

Abbott Laboratories, Inc.'s Perspective on *B.t.*

Robert Fusco, Abbott Laboratories Inc., Manager, Forestry-Vector Field R&D, Mifflintown, Pennsylvania

The commercial use of *B.t.* (*Bacillus thuringiensis*) for forest and agricultural pests has evolved over three decades and continues to evolve at a rapid pace especially in the area of optimized, higher potency formulations for forestry aerial applications and in agricultural field crops, the *B.t.*-transgenic plants. The *B.t.*-transgenic plants are genetically engineered plants designed to produce *B.t.* toxic proteins in the plant tissue. It is the fastest developing technology in agriculture today. Millions of acres of *B.t.*-cotton, *B.t.*-corn, and *B.t.*-potatoes will be planted in the U.S. this year, and it is predicted by the year 2001, over 50 percent of the acreage planted to these crops will be with

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B.t.-transgenic seed. It is unlikely that slow-growing *B.t.*-transgenic trees capable of producing multiple toxins necessary to control the major forest pests will be developed in the near future.

The evolution of *B.t.* from a low-potency, crude, primary powder preparation, which was used only on vegetable crops to optimized high-potency formulations designed to be sprayed ULV by aircraft against forest pests, has occurred because of significant advances in technology involving the culture, mass production, formulation, and application of this unique bacteria. Along with these technological advances came a dramatic improvement in the quality of *B.t.* chemical insecticides. The following is a brief discussion of some relatively new developments in *B.t.*'s use in forestry and other *B.t.* products being used in Public Health.

Recently developed *B.t.* formulations for forestry use:
Foray 48F® is an aqueous-based suspension of *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). It was introduced in 1996 as a result of major breakthroughs in fermentation technology by Abbott Laboratories. In the production of this formulation, the fermentation process is optimized using HPLC (High Performance Liquid Chromatography) and instead of expressing potency in international units (IU's) based on the cabbage looper, *Trichoplusia ni*, they are expressed in Forestry Toxic Units (FTU) based on the gypsy moth bioassays. Other factors which impact *B.t.* activity and which cannot be measured solely by HPLC, including *B.t.* crystal solubility, protein activity, synergy among toxins, number of spores, and *B.t.*-related metabolites, were also optimized. This formulation contains 11,800 FTU's of potency per mg or 48 Billion Forestry Toxic Units per gallon and is designed to be sprayed undiluted at 21-128 oz/acre against the gypsy moth.

Foray 96B® is the first high-potency aqueous formulation of *B.t.k.* designed for undiluted applications on coniferous forests against defoliating Lepidopteran pests. It has been tested extensively in Canada and Europe against various economic pests and is expected to have full registration in 1999 or 2000. It was designed to have greater biological activity at a lower applied volume, i.e., applied in smaller, higher potency droplets, which increases aircraft payload and would lower application costs. Initial tests against spruce budworm have shown reduced *B.t.* feeding inhibition and quicker larval mortality; at 95 BIU/L, <80 micron droplet = LD95. It contains 96 IU/gal (25.4 BIU/L) and is applied at 10-27 oz/acre (.75 -2.0 L/ha).

Novodor®, a product registered for Colorado potato beetle and elm leaf beetle contains the crystal protein toxins produced by *Bacillus thuringiensis* subsp. *tenebrionis*. The strain was improved from the original strain using classical mutation techniques. The strain used in Novodor® produces a bigger crystal, which is directly correlated with enhanced field activity. In 1998, field trials showed that Novodor® was very effective against larvae of the cottonwood leaf beetle, *Chrysomela scripta*, the number one pest on plantation-grown hybrid poplar.

Additional advances in *B.t.* formulations for forestry use are anticipated in the future. Formulations with higher potency enhanced with toxic genes specific for forestry pests are being developed. These formulations are being designed to have increased physical and biological stability, quicker biological activity and enhanced persistence on target foliage.

Other Registered Microbial Pesticides from Abbott Laboratories:

Microorganism	Target Pests	Product
B.t. israelensis	mosquitoes, blackflies	VectoBac®
B.t. aizawai	Lepidoperan larvae	Xentari®
Bacillus sphaericus	stagnant-water Mosquitoes	Vectolex.®

B.t. in Transgenic Plants

John R. Omer, USDA Forest Service

Transgenic plants are one of the latest uses of *Bacillus thuringiensis* (*B.t.*). *B.t.* Cry toxins (naturally occurring insect toxin) can be engineered into plants to exhibit resistance against insects. The *B.t.* gene that is responsible for the production of the lethal protein is isolated. That gene is then removed from the *B.t.* bacterium and inserted into the plant cells. Researchers grow the plant cells into whole plants by a process called tissue culture. The modified plants produce the same lethal *B.t.* protein produced by the *B.t.* bacterium because the plants now have the same gene. Crops that have been developed include: potatoes, field corn, soybeans, and cotton. Many other commercial crops are targeted for *B.t.* insect resistance. Some of the insects that are targeted by this technology include Colorado potato beetle, European corn borer, tobacco budworm, cotton bollworm, and pink bollworm.

Some of the advantages — reduced chemical pesticide use, thus; reduced human and environmental exposure to chemical pesticides; reduced labor and cost; increased productivity.

Some of the risks — development of *B.t.* resistant pests through natural selection; impact on nontarget insects; persistence of toxins in the environment; impact on microbial populations and processes in the soil.

Thermo Trilogy Corporation's Perspective on *B.t.*

Ron L. White, Director Sales and Marketing Thermo Trilogy Corp., Columbia, Maryland

The brief history of Thermo Trilogy is limited to just a short 3-year period in which our organization of less than three million dollars in annual sales has grown into one of the top two-three biorational pesticide companies (sales dollars) in the United States. The core of Thermo Trilogy began as a research group within W.R. Grace Company, and was known as Grace Biopesticides Division. Two developmental projects that the Grace Biopesticide group completed were the commercialization of Neem and the development of fungi for disease control. In the spring of 1996, Thermo Electron acquired the assets of Grace's Biopesticide Division and renamed the organization Thermo Trilogy Corporation. A short 10 months later, Thermo Trilogy acquired the assets of another biopesticide company named "Biosys". The marketing focus was the manufacturing and selling of beneficial nematodes, baculovirus, and pheromone technology. This acquisition also provided an international presence with the addition of an UK organization known as AgriSense LTD. Later in the fall of 1997, Thermo Trilogy completed its last acquisition by purchasing the sprayable technology of Novartis' worldwide *B.t.* business and manufacturing facilities located in Wasco, California. Today, Thermo Trilogy manufactures and offers for sale over 100 different biorational products targeted at agricultural, consumer/retail, forestry, ornamental, and PCO markets throughout the world.

One major product segment for Thermo Trilogy involves the manufacturing and selling of *B.t.* products for agricultural, forestry, and retail markets. A breakout of Thermo's *B.t.* product line by segment is listed as follows:

Agricultural markets (B.t.k.): Agree®, Javelin®, CoStar®, Able®, Design®

Forestry markets (B.t.k.): Thuricide® 48LV, Thuricide® 76LV

Vector markets (B.t.i.): Teknar® HPC

Consumer markets (B.t.k.): Thuricide® HPD

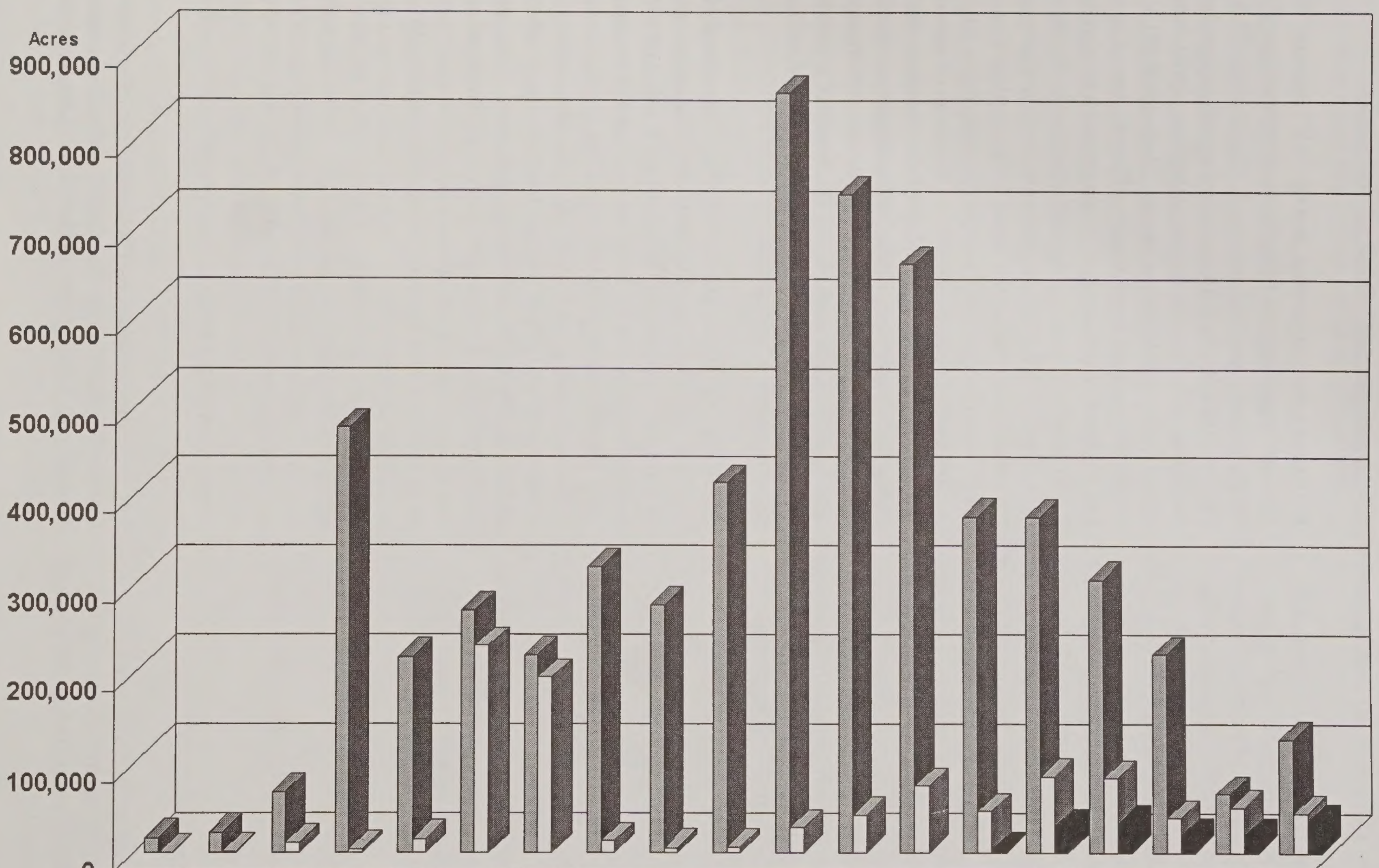
All Thermo Trilogy *B.t.* products are manufactured. Thermo Trilogy's QC personnel at our manufacturing facilities in California complete bioassays before any product is packaged for customer shipment.

Thermo Trilogy's offering to the forestry market is a *B.t.k.* product manufactured and sold under the brand name of "Thuricide® 48LV". The 48LV relates to the number of BIU's per gallon of product, and the "LV" denotes "Low Volume" aqueous spray formulation developed specifically for the forestry aerial use segment. Thuricide® 48LV is a microbial insecticide with an active ingredient based on a bacterium, *Bacillus thuringiensis* Berliner, which is active against many lepidopterous larvae. Thuricide® 48LV acts specifically against many species of lepidopterous larvae, and has no direct effect on eggs. It is a stomach poison for the larval stage only, and has no contact action to the insect. Thuricide® 48LV produces delta-endotoxins which causes gut paralysis soon after the insect feeds on plant tissue and *B.t.k.* Insect larvae usually die within one to five days after feeding on *B.t.*/Thuricide® 48LV. Spectrum of activity for *B.t.k* products such as Thuricide® have demonstrated the ability for affecting more than 200 lepidopterous species in their larval stages. The original formulation of Thuricide® was developed and marketed by the Sandoz Corporation during the 1970's and 80's throughout North America in a less concentrated formulation.

Application timing for Thuricide® 48LV is extremely important in assuring a successful treatment. The major criteria affecting the appropriate timing for *B.t.k.* are: 1) the shoot or foliage development of the target trees, and 2) the development stage of the target insect. Initiating application as early as possible, consistent with the aforementioned criteria, also has the beneficial effect of minimizing pre-spray defoliation and improving post-spray foliage protection. For gypsy moth applications: it is best to spray gypsy moth when most larvae have hatched and are between the first and third instar stage. Foliage development (leaf expansion) should be at least 25 to 50 percent to provide adequate leaf area for the deposit of Thuricide® 48LV. Application of product can be applied in one single treatment or split into two separate application treatments to impact multiple egg hatches occurring on the leaf foliage. Currently, Thuricide® 48LV is offered in 53-gallon drums or in truckload tanker configurations to service the aerial applicators in North America and Europe.

For further information on Thermo Trilogy products, Contact Thermo Trilogy Customer Service Department at 800-847-5620 during our business hours Monday through Friday.

B.t. Use for Gypsy Moth Control
Totals include all ownerships



	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Supression	16,963	22,437	67,324	475,898	218,315	270,226	219,323	318,849	276,050	413,318	851,532	738,313	659,536	375,368	374,922	304,489	220,474	65,447	126,206
Eradication	40	1,470	11,450	3,980	15,550	230,120	195,030	13,320	5,500	5,980	27,870	41,430	74,170	46,150	83,950	83,070	38,640	50,180	43,710
Slow-the-Spread														3,200	29,060	32,530	18,930	18,740	34,530

Suppression: Treatments designed to prevent damage in the generally infested areas of the Northeast.
Eradication: Treatments designed to eliminate the gypsy moth outside of the generally infested areas.
Slow the Spread: Treatments made within a specially designated buffer zone between the generally infested and the eradication areas.

The Coupon

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